ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

Enhancing Toxicity Prediction with MLinvitroTox: Prioritizing Unidentified Compounds in Environmental Samples Based on Hazard Assessment

Master Thesis

Robin Bosshard, 16-915-399

October 16, 2023

Supervisors: Prof. Dr. Fernando Perez-Cruz, Dr. Eliza Harris, Lili Gasser (SDSC)
Dr. Kasia Arturi (Eawag)

Department of Computer Science, ETH Zürich

Abstract

This thesis enhances the MLinvitroTox framework, which predicts the toxicity of unknown chemical compounds from High-Resolution Mass-Spectrometry (HRMS/MS) fragmentation spectra data. This framework forecasts the most hazardous compounds in environmental samples, circumventing the need for resource-intensive analytical chemical identification. The predictivity is evaluated on SIRIUS molecular fingerprints from MassBank spectra data. However, the machine learning models are trained on molecular fingerprints derived from chemical structure and utilized in vitro toxicity data from ToxCast/Tox21. We have developed pytcpl, a Python-based processing pipeline that extends its applicability to the latest toxicity data. We have leveraged datasets spanning various assay endpoints that encompass a wide range of toxicity aspects. The individual machine learning models achieve an average balanced accuracy of todo:X when predicting binary toxicity and demonstrate effectiveness when validated using MassBank spectra data. Furthermore, a user-friendly web app was created to facilitate interaction with this framework.

Acknowledgments

First and foremost, I would like to thank Prof. Dr. Fernando Perez-Cruz from the Swiss Data Science Center (SDSC) for granting me the opportunity to work on this fascinating project. His support has been invaluable.

I would like to express my sincere gratitude to my supervisor Dr. Kasia Arturi from Swiss Federal Institute of Aquatic Science and Technology (Eawag) and my supervisors Dr. Eliza Harris, Lili Gasser from SDSC for their numerous discussions, patience and valuable insights. Without their help, this thesis would not have been achievable.

Additionally, I would like to acknowledge Prof. Dr. Juliane Hollender from Eawag for her support throughout the project and for the enlightening experience of visiting the Eawag labs.

Furthermore, my gratitude goes out to Jason Brown, Feshuk Madison, and Katie Paul Friedman for their participation in discussions concerning the technical aspects of the tcpl pipeline and the ToxCast database.

Lastly, I extend a special thank you to my family and friends for their unconditional support throughout my academic journey.

Contents

Chapter 1

Introduction

1.1 The Challenge of Environmental Pollution

Over the past few decades, the upsurge in environmental pollution by chemical compounds has been driven by industrial processes, agricultural methods, consumerism and various other contributing factors. Although these chemicals are integral for many products and have the potential to improve the comfort of modern society, they can also pose risks and adversely affect both human health and the environment, either acutely or chronically. Toxic substances threaten wildlife but also make air, soil, drinking water and food supply less safe.

Nations worldwide maintain comprehensive chemical regulations[1], however, it is anticipated that global chemicals production will double by 2030 [1]. Moreover, the widespread utilization of chemicals, including their inclusion in consumer goods, is expected to expand further. Even though there are over 275 million known chemical compounds registered by the Chemical Abstracts Service [2], merely a tiny fraction of them undergo close monitoring via target analytical approaches and even less is known about their toxicity profiles and negative health effects on organsims. Refer to Table 1.1 for an overview of omnipresent water pollutants.

In light of the rapidly evolving chemical landscape, there is an increasing demand for future-proof, robust measurement and modeling methods. These methods are crucial for evaluating the toxicity of chemicals, facilitating informed risk-based decision-making even when data on hazards and exposures are limited. It is worth noting that the need for adaptable approaches in chemical safety and sustainability efforts must also prioritize cost-efficiency and gain widespread acceptance among regulatory bodies, industry stakeholders, and the general public. For instance, the EU has introduced the 8th Environment Action Programme, as outlined in its European Green Deal [4], to provide direction for European environmental policy until the year 2030. This

[1]For instance, REACH, short for Registration, Evaluation, Authorisation, and Restriction of Chemicals, is an EU regulation aimed at improving chemical safety and allocating risk management responsibilities to companies operating in various sectors.

Table 1.1: Examples of ubiquitous water pollutants. Table 2 adapted from [3].

Origin/Usage	Class	Examples	Related Issues
Industrial Chemicals	Solvents Intermediates Petrochemicals	Tetrachloromethane Methyl-t-butylether BTEX	Hepatotoxic Drinking-water-quality Cancer
Industrial Products	Additives Lubricants Flame Retardants	Phthalates PCBs PBDEs	Endocrine disruptors Biomagnification
Consumer Products	Detergents Pharmaceuticals Hormones	Nonylphenol ethoxylates Antibiotics Ethinyl estradiol	Endocrine effects Bacterial resistance Feminization of fish
Biocides	Pesticides	DDT	Toxic effects and persistent metabolites
Natural Chemicals	Heavy Metals Inorganics Taste and Odor Hormones	Lead, mercury Arsenic, fluoride Geosmin Estradiol	Organ damage Drinking-water-quality Feminization of fish
Disinfection & Oxidation	Disinfection by-products	Haloacetic acids, Bromate	Drinking-water-quality
Transformation Products	Metabolites from all above	Metabolites of perfluorinates	Bioaccumulation

program reinforces the EU's ambitious goal of sustainable living within planetary limits, with a forward-looking vision that extends to 2050. Central to this vision is a zero-pollution commitment, encompassing air, water, and soil quality. In 2021, the European Commission introduced a sustainability-focused chemicals strategy [5], which aligns with the EU's zero-pollution ambition. This strategy not only enables the evaluation of the safety and sustainability of emerging compounds but also aims to reduce existing concerning substances, such as *per- and polyfluoroalkyl substances (PFAS)*, through substitution or phasing out wherever feasible. In parallel, the *U.S. Environmental Protection Agency (EPA)* shares a similar scientific consensus and is at the forefront of assessing the potential impacts of chemicals on human health and the environment. Leveraging advanced toxicological methods, EPA actively promotes risk reduction efforts through its own Chemical Safety for Sustainability National Research Program. This program builds upon the achievements of research initiatives like *ToxCast/Tox21*[2] and the Endocrine Disruptor Screening Program in the 21st Century (EDSP21), demonstrating a commitment to advancing chemical safety on a global scale.

[2] https://www.epa.gov/chemical-research/exploring-toxcast-data

1.2 The Imperative for Prioritization and Toxicity Assessment

Modern analytical techniques, including *high resolution mass spectrometry (HRMS/MS)*, are gaining significance across various domains such as metabolomics, drug discovery, environmental science and toxicology [6].

In environmental monitoring, the application of nontarget HRMS/MS has notably improved the capacity to detect possibly thousands of contaminants in a single sample. The instrument generates complex spectra that provide information about the masses and fragmentation patterns of compounds present within the sample as illustrated in Figure 1.1. Often, only a minority of these molecules can be definitively identified, while the majority remains unidentified, resulting in their classification into two categories:

- **Identified compounds** are substances for which their chemical structure and properties have been determined and confirmed using additional analytical techniques. These compounds are precisely characterized and can be linked to existing databases or reference spectra, enabling the retrieval of information about their toxicity and other relevant characteristics.
- **Unidentified compounds**, on the other hand, are substances that are detected but lack definitive characterization in terms of their chemical identity, structure, or properties, including its toxicity. Unidentified compounds are observed as peaks or features in these spectra, but their specific chemical attributes remain unknown. Further examination of these compounds is necessary, but it entails a substantial investment of time and resources, emphasizing the importance of prioritization.

When it comes to prioritizing unidentified compounds for further in-depth testing and identification, the standard approach has been to rely on signal intensity from fragmentation data. However, this approach tends to fall short in delivering an accurate assessment of environmental exposures because the signal intensity may not relate proportionally to the compound's concentration in the sample. Furthermore this approach overlooks the toxicological factors essential for prioritizing compounds with concers related to environmental hazards. As a result, substances with the potential for severe ecological consequences, such as endocrine-disrupting compounds, often go undetected because of their low abundance, even though they exhibit high levels of toxicity. Hence, a pressing need exists for alternative approaches to prioritize unidentified nontarget HRMS/MS signals based on their hazard potential. By incorporating relevant toxicity factors into the equation:

$$\text{Risk} = \text{Hazard} \times \text{Exposure} \tag{1.1}$$

we augment the capacity to make well-informed decisions when evaluating the environmental risk associated with chemicals.

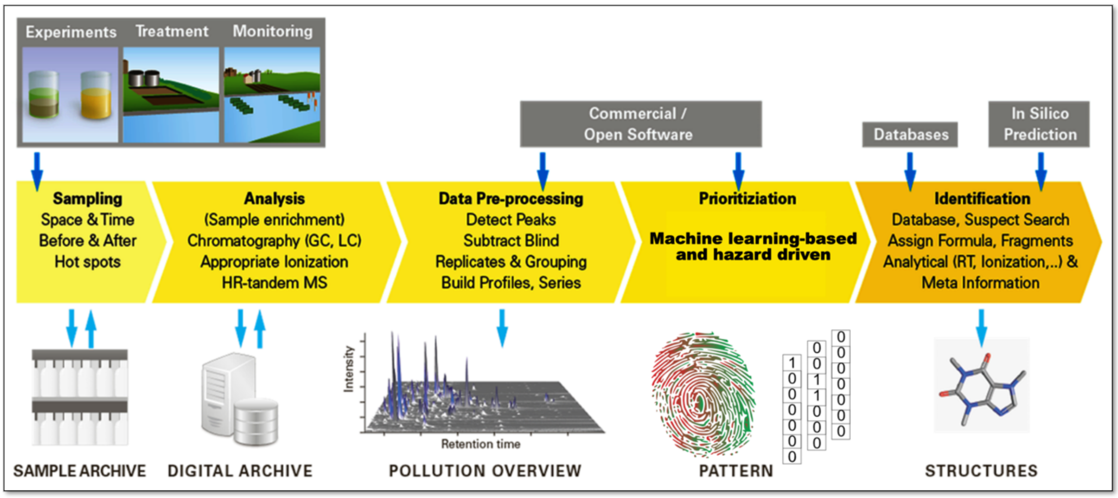


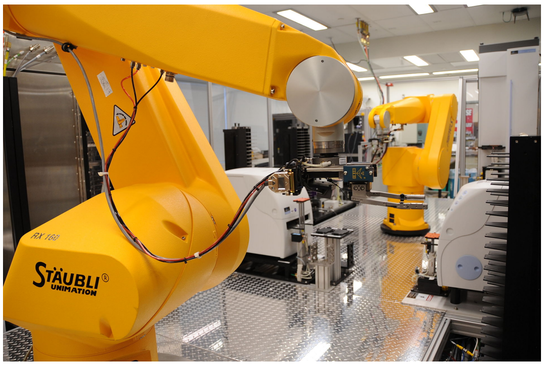
Figure 1.1: Schematic of the workflow used for nontarget HRMS/MS screening of environmental samples, featuring a customized prioritization step. Adapted from Figure 1 in the original source [7].

1.3 Unlocking the Potential of High-Throughput Screening and Machine Learning in Toxicity Prediction

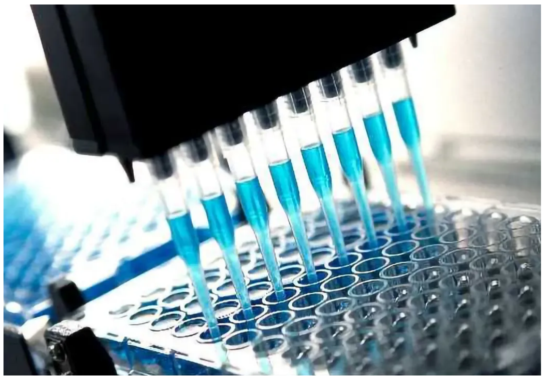
In the past few years, the use of machine learning methods has emerged as a transformative force in the field of *in vitro* toxicology, particularly in the realm of high-throughput toxicity prediction. *High-throughput screening (HTS)* has revolutionized the way toxicity is assessed by allowing thousands of *in vitro* bioassays to be conducted efficiently. This high-throughput approach, coupled with advancements in robotics and automated analysis, has generated large volumes of toxicity data, paving the way for more comprehensive assessments of chemical compounds. Alongside the rise of machine learning, this advancement has facilitated the creation of predictive models, known as *Quantitative structure-activity relationship (QSAR)* models. These models are capable of forecasting bioactivity or compound toxicity based on their physico-chemical properties or molecular descriptors [8]. As they are trained on extensive datasets containing toxicity information, these models can learn the underlying patterns and relationships between chemical structures and target toxicity. With this capability, they can predict the toxicity of new compounds, even when these substances themselves have not undergone laboratory testing. This approach holds the potential to substantially decrease the time and expenses linked to initial toxicity pre-assessment, and it plays a pivotal role in determining which compounds should undergo more in-depth testing.

1.4 MLinvitroTox: A Novel Approach

In response to the pressing need for a more hazard-driven and inclusive assessment of environmental contaminants, *Arturi et al.* introduced *MLinvitroTox* [11], an innovative



(a) A robot arm retrieves assay plates from incubators and places them at compound transfer stations or hands them off to another arm that services liquid dispensers or plate readers. Efforts in the automation, miniaturization and the readout technologies have enabled the growth of HTS. Image obtained from [9].



(b) Modern microtitre assay plates consist of multiples of 96 wells, which are either prepared in the lab or acquired commercially from stock plates. These wells are filled with a dilution solvent, such as *Dimethylsulfoxide (DMSO)*, along with the chemical compounds intended for analysis. Image obtained from [10].

Figure 1.2: High-Throughput Screening (HTS)

machine learning framework. This framework is part of a broader pipeline named *EXPECTmine*, which incorporates the complementary exposure aspect within the risk assessment process. The primary objective of this thesis is to collaborate with the authors to further enhance and advance this framework. MLinvitroTox leverages molecular fingerprints extracted from fragmentation spectra, marking a significant change in how the toxicity of the myriad unidentified HRMS/MS features is forecasted. MLinvitroTox follows a similar training approach as traditional QSAR models, using supervised classification models trained with molecular fingerprints derived from chemical structures. However, during the application phase, the input to the machine learning model consists of molecular fingerprints generated from experimentally measured mass-spectrometry fragmentation spectra using *SIRIUS* and *CSI:FingerID* [12]. SIRIUS is a software package for annotating small molecules from nontarget HRMS/MS data, while CSI:FingerID is a machine-learning tool employed by SIRIUS to predict molecular fingerprints from fragmentation spectra. Utilizing streamlined machine learning methodologies, MLinvitroTox forecasts chemical toxicity for a wide range of compounds. This analysis covers more than 300 target-specific assay endpoints, drawing data from ToxCast/Tox21 datasets. Subsequently, the toxicity predictions generated by the framework are employed to prioritize compounds, with the flexibility to emphasize specific aspects of toxicity profiles.

1.5 Objectives and Significance

The main objective of this thesis is to contribute to the development of an efficient MLinvitroTox framework for predicting compound toxicity across multiple endpoints. The goal is to enhance the integration of MLinvitroTox by creating an automated pipeline in the `Python` programming language. This pipeline is designed to efficiently address the inherent complexities associated with modeling and processing heterogeneous datasets. In this context, the primary focus is on elevating the quality of curating and preparing toxicological data, with a particular emphasis on streamlining the entire process. This process begins with raw concentration-response series data and ultimately leads to the generation of conclusive toxicity predictions. The ultimate output is expected to comprise *toxicity fingerprints* that encapsulate the predicted toxicity from HRMS/MS environmental samples for the relevant endpoints of interest. These generated toxicity fingerprints will offer crucial insights for the prioritization process, aiding in the identification of the most hazardous compounds present in environmental samples.

One notable contraint of the existing framework lies in its binary *hitcall* when predicting the toxicity of specific endpoints. It categorizes compounds as either toxic or non-toxic without accounting for variations in toxicity severity. In the long term, it is essential to adopt a more refined approach that can capture the nuanced continuum of toxicity. This thesis endeavors to overcome this limitation by developing a pipeline capable of forecasting toxicity across numerous endpoints, employing continuous hitcalls.

1.6 Thesis Structure

In the course of progressing through the subsequent chapters, insights will be provided into the materials and methods employed, focusing on the technical intricacies involved in the preparation of ToxCast/Tox21 toxicity data and their transformation into suitable inputs for the machine learning pipeline. This foundational work will establish the basis for the upcoming chapters, which will showcase the potential of MLinvitroTox. Furthermore, the framework's effectiveness is demonstrated through the validation of real-world mass spectral data from *MassBank* [13], and the examination of the implications of this research is carried out.

Chapter 2

Background

This chapter is vital for understanding the following sections of this thesis as it provides some foundational background information in toxicity testing.

2.1 Toxicity Testing: From In Vitro Assays and Molecular Fingerprints to Predictive Models and Beyond

With the ever-growing amount of chemical compounds entering the environment, traditional experimentation methods face limitations concerning cost and time constraints. Additionally, ethical concerns arise regarding the use of animal trials in *in vivo* experiments.

In 2007, the *U.S. National Academy of Sciences* introduced a visionary perspective and published a landmark report, titled as *Toxicity Testing in the 21st Century: Vision and Strategy*. This report promoted a transition from conventional, resource-consuming animal-based *in vivo* tests to efficient high-throughput *in vitro* pathway assays on cells. This transition paved the way for the realm of HTS, where a multitude of *in vitro* bioassays can be executed, complementing and improving chemical screening. This transformation is made possible by advancements in robotics, data processing, and automated analysis. As a result, this synergy has led to the generation of extensive toxicity datasets like ToxCast/Tox21.

HTS datasets, including ToxCast and other sources, have opened the door to promising applications of machine learning in predictive computational toxicology. These predictive models can be developed to screen environmental samples with limited availability of toxicity data, allowing for the prioritization of further testing efforts. Such models often forecast toxicity using QSARs, which are based on descriptors encoding chemical structures like molecular fingerprints. 1D-Molecular fingerprints encode compound molecules as fixed-length binary vectors, denoting the presence (1) or absence (0) of specific substructures or functional groups, visualized in 2.1. Typically, fingerprints use *SMARTS* strings, as an extension of *SMILES* strings, to encode the underlying

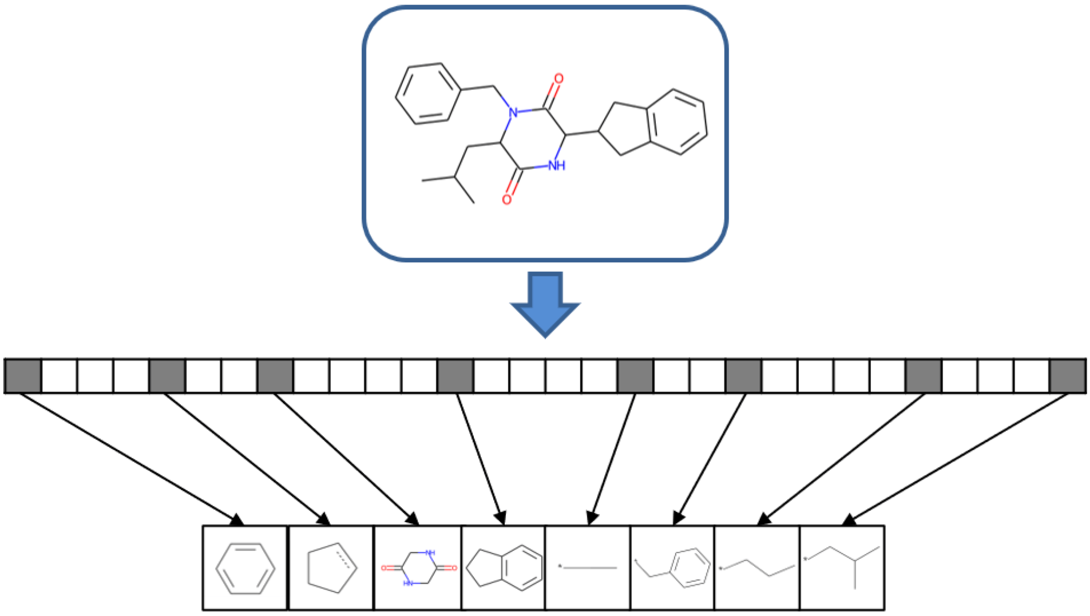


Figure 2.1: Schematic of a molecular fingerprint for a fictional chemical. Each bit position accounts for the presence or absence of a specific structural fragment. Bit positions are set on (set to 1, gray) if the substructure is present in a molecule, or set off (set to 0, white) if it is absent. Figure 1 adapted from [14].

substructural patterns within molecules. While SMILES is a widely accepted notation system for representing chemical structures, there can be variations in how different sources generate SMILES strings. These variations can include the presence or absence of hydrogens, different ways of representing aromatic rings, variations in chemotypes, and tautomer representations. SMILES strings for the same chemical can differ due to these variations. To ensure consistency and deterministic computation, chemists often normalize SMILES before use, ensuring adherence to a common set of rules for computational analysis.

Once generated, molecular fingerprints can be used for various cheminformatics tasks. For example, they can be compared to identify structurally similar compounds, used as input for machine learning models to predict properties or activities.

To go one step further, SIRIUS employs CSI:FingerID, a method that directly predicts various fingerprint types from HRMS/MS fragmentation spectra. CSI:FingerID utilizes machine learning techniques, encompassing linear Support Vector Machines and Deep Learning, to predict an array of fingerprints, including CDK Substructure, PubChem CACTVS, Klekota-Roth, FP3, MACCS, ECFP2, and ECFP4 fingerprints.

The utilization of molecular fingerprints for *in vitro* toxicity prediction is based on the assumption that molecular toxic effects result from interactions between distinct chemical components and receptors during a *molecular initiating event (MIE)*. On a larger biological scale, the MIE can set a sequential chain of causally linked *key events (KE)* in motion. This occurs at different levels of biological organisation from within cells to potentially culminating in an *adverse outcome pathway (AOP)* at the organ or organism level, as depicted in Figure 2.2. The mechanistic information captured in AOPs reveal how chemicals or other stressors cause harm, offering insights into disrupted biological processes, potential intervention points but also guide regulatory decisions on next generation risk assessment and toxicity testing. The AOP framework is an analytical construct that allows an activity mapping from the presence or absence of certain molecular substructures encoded in chemical descriptors to the target mechanistic toxicity. Finally, when monitoring disruptions in toxicity pathways, physiologically based pharmacokinetic (PBPK) models can be leveraged to extrapolate *in vitro* findings to human blood and tissue concentrations [15].

It is important to emphasize that the predictions from HTS bioassays portray molecular toxicity events only at a cellular level, and their translation to adverse outcomes at higher organism levels is not necessarily guaranteed. As the scale shifts from the cellular to the organism level, the confidence in these relationships may decrease.

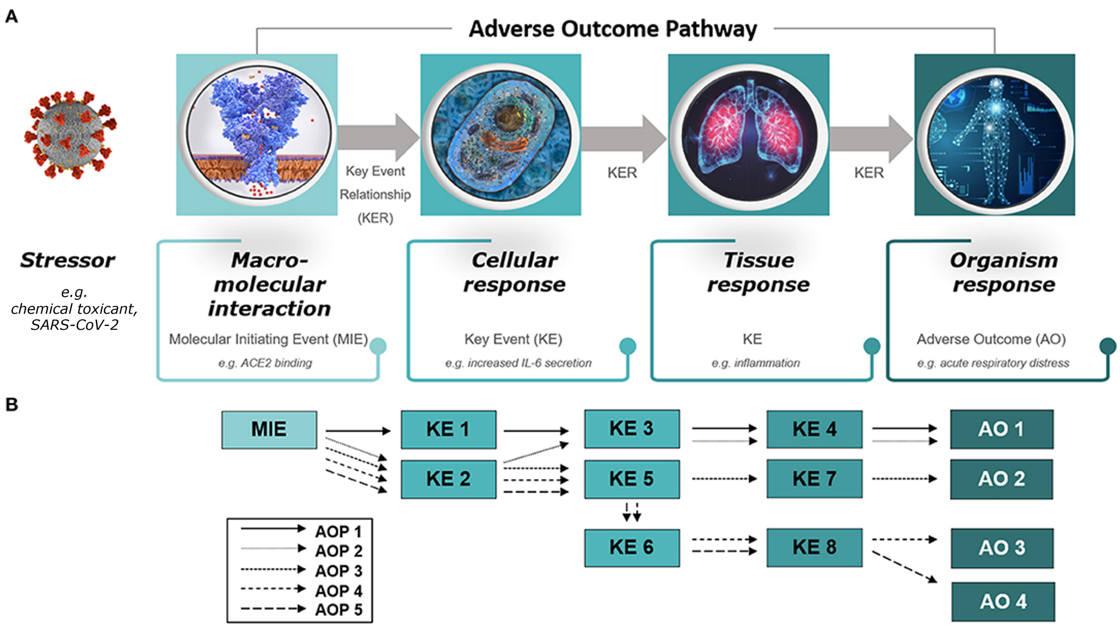


Figure 2.2: Diagram of (A) an adverse outcome pathway (AOP) and (B) an AOP network. (A) An AOP starts with a molecular initiating event (MIE), followed by a series of key events (KEs) on different levels of biological organization (cellular, tissue, organ) and ends with an adverse outcome (AO) in an organism. The stressor is not part of the AOP itself. Figure 1 adapted from [16]

2.2 Chemical Target Toxicity vs. Cytotoxicity

Consider a hypothetical scenario in which a chemical undergoes testing in a bioassay that assesses toxicity by measuring the activation of a *reporter gene* within a cell. The reporter gene encodes a detectable protein, and its activation is triggered by the chemical binding to a specific receptor, the key focus of the assay endpoint. While it might seem logical that an increase in chemical concentration would result in a higher chemical toxicity signal, this assumption does not hold true in general. At elevated concentrations, the chemical can become *cytotoxic*, causing harm to the cells and ultimately leading to cell death. Consequently, this can lead to a decrease in the activation of the reporter gene and a subsequent reduction in the signal, indicating a decrease in bioactivity. For a visual representation, please refer to Figure 2.3. Considering this situation, chemical toxicity can manifest in various forms, categorizing into two primary groups [17]:

- **Specific toxicity** occurs when a chemical interacts with and interferes with a specific biomolecular target or pathway, manifesting as effects like receptor agonism/antagonism or enzyme activation/inhibition. This thesis primarily focuses on specific toxicity, which is often the desired signal inteneded for detection through a target assay endpoint. However, it is essential to recognize that data processing must also take into account the following:
- **Non-specific toxicity (Cytotoxicity and cell stress)** involve broad disruptions of the cellular machinery, including reactions with DNA as well as processes like apoptosis, oxidative stress and mitochondrial disturbance. Cell viability can be evaluated either individually or concurrent with the target bioassay endpoint. For instance, one approach involves evaluating the cell viability by determining the proportion of live cells within a population. This is achieved using a fluorescent dye that selectively enters living cells, as it cannot permeate the membranes of deceased cells, resulting in fluorescence intensity directly reflecting cell viability.

An associated phenomenon is referred to as the *cytotoxicity burst* [17], in which the expected specific toxicity interferes with non-specific cellular stress responses that may become overly activated within a critical range of toxicant concentration. As the concentration of the toxic substance approaches levels that induce cell death, the signal associated with the presumably specific toxicity of a target assay endpoint becomes increasingly mixed with signals stemming from non-specific responses [18]. Only from the observed responses of the target assay endpoint it can not be deduced what are the specific and non-specific shares in the measured signal.

Referred to as *false positive* hitcalls, these are associated with compounds where the activity response surpasses the efficacy cutoff mainly because of non-specific toxicity. Nevertheless, in many research contexts, there exists a specific interest in pinpointing specific toxicity [19]. This becomes crucial for identifying the molecular initiating event and understanding the adverse outcome pathway. Solely based on the observed signal, the challenge arises in differentiating true positives, where the compound exhibits specific toxicity without cytotoxicity interference, from false positive hitcalls. This

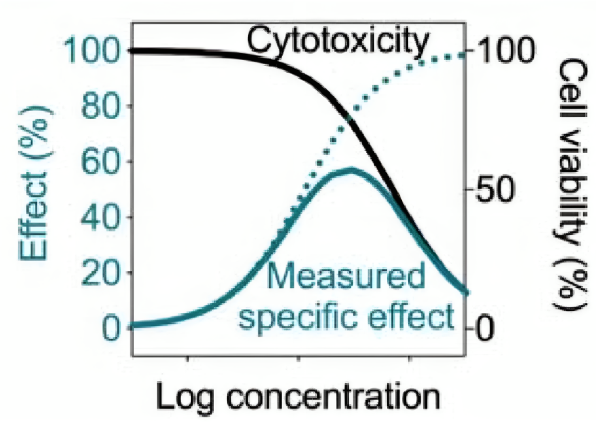


Figure 2.3: Example of a bioassay response with cytotoxicity interference. The dotted line shows the theoretical specific toxicity effect but due to non-specific cytotoxicity (black line is cell viability), the measured effect may have an inverted U-shape within the tested consentration range. The measured specific effect may also be influenced by the presence of the cytotoxicity burst phenomenon, which can lead to a non-specific exponential growth phase before the subsequent decline in the effect curve. Figure 7.8 from [18].

introduces significant uncertainty in the reported activity hitcalls.

Nonetheless, the ToxCast pipeline is deliberately structured to minimize the occurrence of *false negative* hitcalls. The original pipeline employs a fairly inclusive risk assessment approach, ensuring that compounds with ambiguous toxicity potential are more likely to be rated as active rather than inactive. Moreover, the toxicity assessment process within this pipeline lacks proper mechanisms to differentiate between activity arising from specific and non-specific chemical toxicity.

Although not the central emphasis of this study, we investigate the possibility of reducing potential overestimation of positive hitcalls attributed to suspected non-specific component in the reported activity. This is achieved by comparing potency concentrations between the target assay endpoints and the corresponding viability or burst assay endpoints, which quantify cytotoxic cell loss or cell stress, respectively. If the probabilities indicate that a crucial potency concentration from the cytotoxicity assay endpoint is lower than that of the target assay endpoint, previously identified false positive hitcalls can be reduced by a factor reflecting the potential impact of cytotoxicity interference.

Chapter 3

Related work

Recent advancements in ML-based prediction of toxicity endpoints, were summarized in [20] and it was found that teh progresses are driven primarily by the efforts in drug discovery.

The recent developments in machine learning for predicting toxicity endpoints were outlined in [20], with an observation that these advancements are primarily driven by the progress made in the field of drug discovery. The study underscores that machine learning approaches demonstrate varying performance levels across diverse toxicity endpoints, with commonly studied ones including cardiotoxicity, mutagenicity, hepatotoxicity and acute oral toxicity,but also those endpoints from the popular Tox21 data challenge [21]. The ability to predict toxicity depends significantly on the characteristics of the datasets, including differences in complexity, class distribution, and the chemical space they encompass, making it challenging to directly compare algorithm performance.

A recent study [22] explores the coverage of large-scale datasets used in machine learning for biomolecular structures, revealing their limitations in representing the full range of known structures. As the chemical space is vast, it is questionable whether the toxicity training data is an informative subset to the true distribution aimed to learn, directly challenging the fundamental assumption in machine learning. The study underscores the importance of taking into account the coverage of chemical space when assessing the effectiveness of machine learning models. In this thesis, the coverage of the chemical space was not specifically assessed, as the focus was on the performance of the models and their ability to generalize to unseen data.

Similar to MLinvitroTox, MS2Tox [23] represents another machine learning approach within the realm of predicting ecotoxicological hazards for unidentified compounds through nontarget HRMS/MS analysis. Both approaches adopt a common strategy of building their ML models based on molecular fingerprints derived from chemical structure, used to make predictions on environmental samples, utilizing fingerprints from fragmentation spectra calculated by SIRIUS+CSI:FingerID. However, ML2Tox diverges in terms of the toxicity data employed for training and testing, with its focus on toxicity data concerning *in vivo* fish lethal concentrations from CompTox [24]. This is in contrast to MLinvitroTox, which relies on *in vitro* toxicity data from ToxCast/Tox21. Additionally, unlike MLinvitroTox, which exclusively relies on molecular fingerprints and does not utilize other physicochemical properties, MS2Tox incorporates the molecular mass of the compound as an additional feature.

In a systematic investigation using Tox21 data [25], the impact of various modeling approaches on predictive toxicology were explored, with a focus on model performance and explainability trade-offs. The study found that endpoints with higher predictability, characterized by lower data imbalance and larger datasets, performed well regardless of the modeling approach or molecular representation. For less predictable endpoints, simpler models like Linear Regression performed similarly to complex ones, thereby emphasizing the importance of balancing predictability and interpretability. Moreover this study suggests consensus modeling and multi-task learning to enhance predictability and model performance across endpoints. In this thesis, the goal was established to not to overlook simpler models due to their higher interpretability and comparable performance. As recommended, no further explorations were conducted regarding the various molecular representations, and instead, a fixed set of molecular fingerprints was employed as the initial input features, with feature selection being applied to reduce the number of relevant features. Furthermore, a consensus modeling approach was adopted, where the final predictions are obtained by averaging the predictions across assay endpoints sharing the same attributes, including mechanistic and biological target.

Chapter 4

Material and Methods

4.1 Toxicity Data and Processing

4.1.1 ToxCast invitroDB v4.1

The most recent release of the ToxCast's database, referred to as *invitroDBv4.1*, serves as a source of an extensive collection of HTS toxicity data (Ĩ00 GB). This database encompasses information on a total of 10 196 compounds, selectively tested across 1485 assay endpoints. Assay endpoints are inherently associated with a single assay in a one-to-many relationship, please refer Figure 4.1 for an overview of the assay annotaion structure.

The assays utilize a range of technologies to assess the impact of chemical compounds on a wide array of biological targets, including individual proteins, nuclear receptor signaling, developmental processes and cellular processes such as mitochondrial health. This resource originates from the collaboration of two prominent institutions: the U.S. EPA through its ToxCast program and the National Institutes of Health (NIH) via the Tox21 initiative. Using data collected from multiple research labs (refer to Table A.1 in the Appendix), this relational database is accessible to the public and can be downloaded[1] by visiting the official ToxCast website.

4.1.2 tcpl v3.0

The primary ToxCast pipeline is effectively managed through the extensive toolkit offered by the *tcpl*[2] package, which includes a variety of tools for high-throughput screening data management. It enables reproducible concentration-response modeling and populates the `MYSQL` database, invitroDBv4.1. The multiple-concentration screening paradigm intends to pinpoint the bioactivity of compounds, while also estimating their efficacy and potency. In Section 4.2, we introduce *pytcpl*, a `Python` reimplementation of

[1] https://www.epa.gov/chemical-research/exploring-toxcast-data, released on Sept 21, 2023
[2] https://github.com/USEPA/CompTox-ToxCast-tcpl

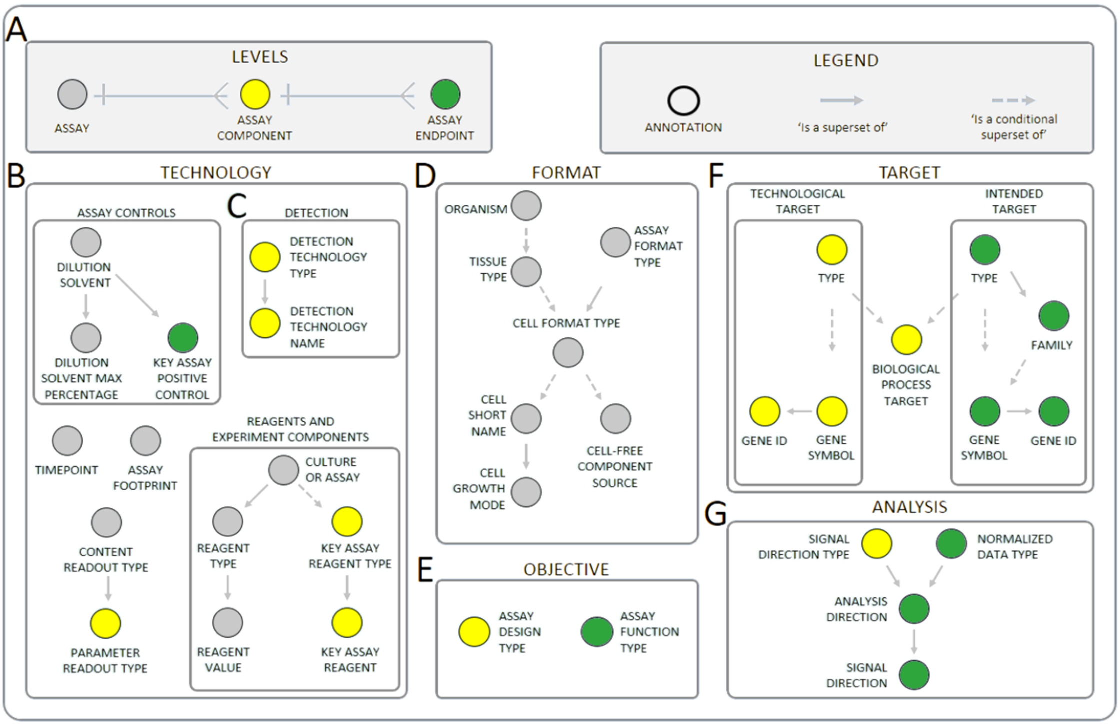


Figure 4.1: Assay endpoints are annotated with (A) assay identification information, (B) design information, (C) target information, and (D) analysis information. Relationships between annotations are either one-to-many or conditional where certain dependencies may not be applicable. Adapted Figure obtained from [26].

the major components that underpin the entire ToxCast pipeline. It should be noted that these components, as presented in the following, are applicable to both tcpl and pytcpl.

4.1.3 Efficacy Cutoff

The evaluation of a compound's bioactivity is significantly influenced by the specific *efficacy cutoff* associated with each assay endpoint, as exemplified for a tested compound in Figure 4.2. It serves as a threshold that differentiates active and inactive compounds, essentially defining the minimum response level of toxicity that is biologically relevant. The process of establishing this threshold involves estimating the noise level in the assay endpoint responses across all tested compounds.

4.1.4 Concentration-Response Series

Each compound c_j tested within an assay endpoint a_i involves the collection of the respective *concentration-response series (CRS)* denoted as CRS_{ij}, showcased in Figure 4.2. A CRS is represented as a set of concentration-response pairs:

$$CRS_{i,j} = \{(conc_{1_{i,j}}, resp_{1_{i,j}}), (conc_{2_{i,j}}, resp_{2_{i,j}}), \ldots, (conc_{n_{\text{datapoints}_{i,j}}}, resp_{n_{\text{datapoints}_{i,j}}})\}$$

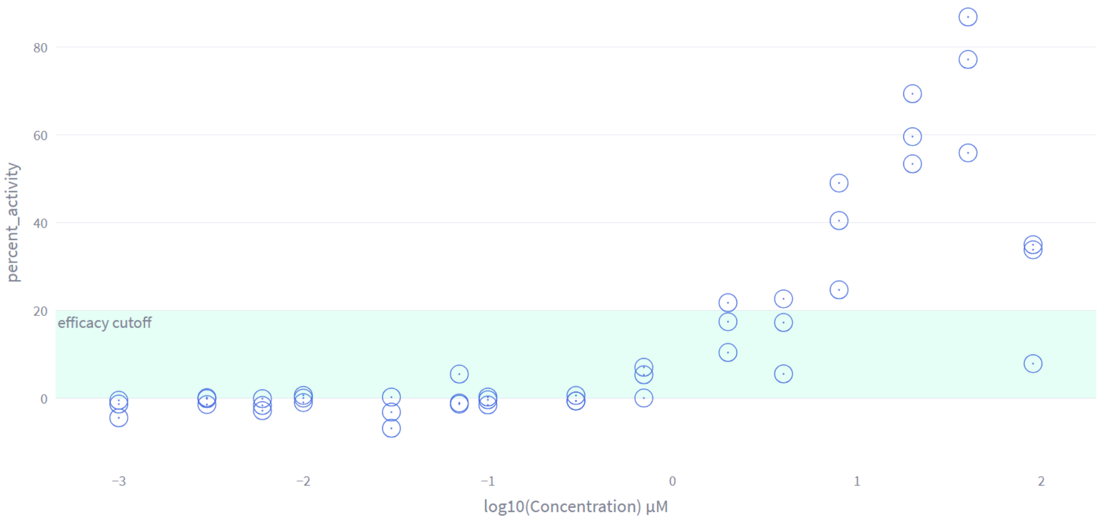


Figure 4.2: The CRS belongs to *Diofenolan* (DTXSID2041884), tested in the assay endpoint *TOX21_ERa_LUC_VM7_Agonist* (aeid=788). The shaded region represents the estimated efficacy cutoff. This particular series comprises a total of $k = 45$ concentration-response pairs and is structured into $n_{conc} = 15$ distinct concentration groups, with each group consisting of $n_{rep} = 3$ replicates.

Table 4.1: Description of Parameters

Quantity	Description
$n_{\text{datapoints}_{i,j}}$	Total number of concentration-response pairs ($\lvert CRS \rvert$)
$n_{\text{groups}_{i,j}}$	Number of distinct concentrations tested
$n_{\text{replicates}_{i,j}}$	Number of replicates for each concentration group
$min_{\text{conc}_{i,j}}$	Lowest concentration tested
$max_{\text{conc}_{i,j}}$	Highest concentration tested

where $n_{\text{datapoints}_{i,j}}$ varies based on the number of concentrations tested.

In practice, concentrations are often subjected to multiple testing iterations, resulting in the distinct concentration groups with replicates. Table 4.1 presents the key quantities associated with an individual CRS when considering a specific assay endpoint a_i and compound c_j. To visualize the variations in these metrics across the complete set of analyzed CRS in this work, please refer to Figure A.1 in the Appendix.

Concentration-response pairs, along with essential sample information such as well type and assay well-plate indices, can be retrieved by combining tables *mc0*, *mc1*, and *mc3* from invitroDBv4.1, which represent the raw data. A special role is assigned to the control wells, which typically contain untreated samples or samples with a known, non-toxic response. They are used as a baseline to normalize the treated samples and account for any background noise in the assay [27]. The concentrations are transformed to the logarithmic scale in micromolar, while the responses are control well-normalized to either fold-induction or percent-of-control activity:

1. **Fold Induction**: is a measure used to quantify how much, for instance, gene expression has changed in response to a treatment compared to its baseline level from the control well set. E.g., if a gene is expressed five times higher in a treated sample compared to the control, the fold induction would be 5.

2. **Percent of Control**: is another way to express the relative change in a activity due to a treatment compared to the control.

4.1.5 tcplFit2

TcplFit2[3] is an extension to tcpl, focused on curve-fitting and hit-calling. The package also offers a flexible and robust fitting procedure, allowing for the use of different optimization algorithms and the incorporation of user-defined constraints. This sets it apart from other open-source CSS modeling packages such as *drc* and *mixtox*, as it is explicitly designed for HTS concentration-response data.

4.1.6 Curve Fitting

All the curve fit models from tcplFit2, as outlined in Table 4.2 and showcased in Figure 4.3, assume that the normalized observations in the CRS conform to a Student's t-distribution with 4 degrees of freedom [28]. The Student's t-distribution has heavier tails compared to the normal distribution, making it more robust to outlier and eliminates the necessity of removing potential outliers prior to the fitting process. The model fitting algorithm in tcplFit2 employs nonlinear *maximum likelihood estimation (MLE)* to determine the model parameters for all available models.

Consider $t(z, \nu)$ as the Student's t-distribution with ν degrees of freedom, where y_i represents the observed response for the i-th observation, and μ_i is the estimated response for the same observation. The calculation of z_i is as follows: $z_i = y_i - \mu_i \exp(\sigma)$, where σ is the scale term. Then the log-likelihood is: $\sum_{i=1}^{n}[\ln(t(z_i, 4)) - \sigma]$, where n is the number of observations.

The *Akaike Information Criterion (AIC)* is used as measure of goodness of fit, defined by the formula: $AIC = -2\log(L(\hat{\theta}, y)) + 2K$, where $L(\hat{\theta}, y)$ is the likelihood of the model given the data and K is the number of model parameters. The model with the lowest AIC value is chosen as the *winning* model. The winning model is then used to estimate the efficacy and potency of the compound. The potency estimates, also called *point-of-departure (POD)* estimates, are derived from the fitted curve, identifying certain *activity concentrations (AC)* at which the curve first reaches certain response levels. Central POD estimates are depicted graphically in Figure 4.4a.

[3] https://github.com/USEPA/CompTox-ToxCast-tcplFit2

Table 4.2: tcplfit2 Model Details

Model	Label	Equations[1]
Constant	constant	$f(x) = 0$
Linear	poly1	$f(x) = ax$
Quadratic	poly2	$f(x) = a\left(\frac{x}{b} + \left(\frac{x}{b}\right)^2\right)$
Power	power	$f(x) = ax^p$
Hill	hill	$f(x) = \frac{tp}{1+\left(\frac{ga}{x}\right)^p}$
Gain-Loss	gnls	$f(x) = \frac{tp}{(1+\left(\frac{ga}{x}\right)^p)(1+\left(\frac{x}{la}\right)^q)}$
Exponential 2	exp2	$f(x) = a\left(\exp\left(\frac{x}{b}\right) - 1\right)$
Exponential 3	exp3	$f(x) = a\left(\exp\left(\left(\frac{x}{b}\right)^p\right) - 1\right)$
Exponential 4	exp4	$f(x) = tp\left(1 - 2^{-\frac{x}{ga}}\right)$
Exponential 5	exp5	$f(x) = tp\left(1 - 2^{-\left(\frac{x}{ga}\right)^p}\right)$

[1] Parameters: a: x-scale, b: y-scale p: (gain) power, q: (loss) power, tp: top, ga: gain AC50, la: loss AC50

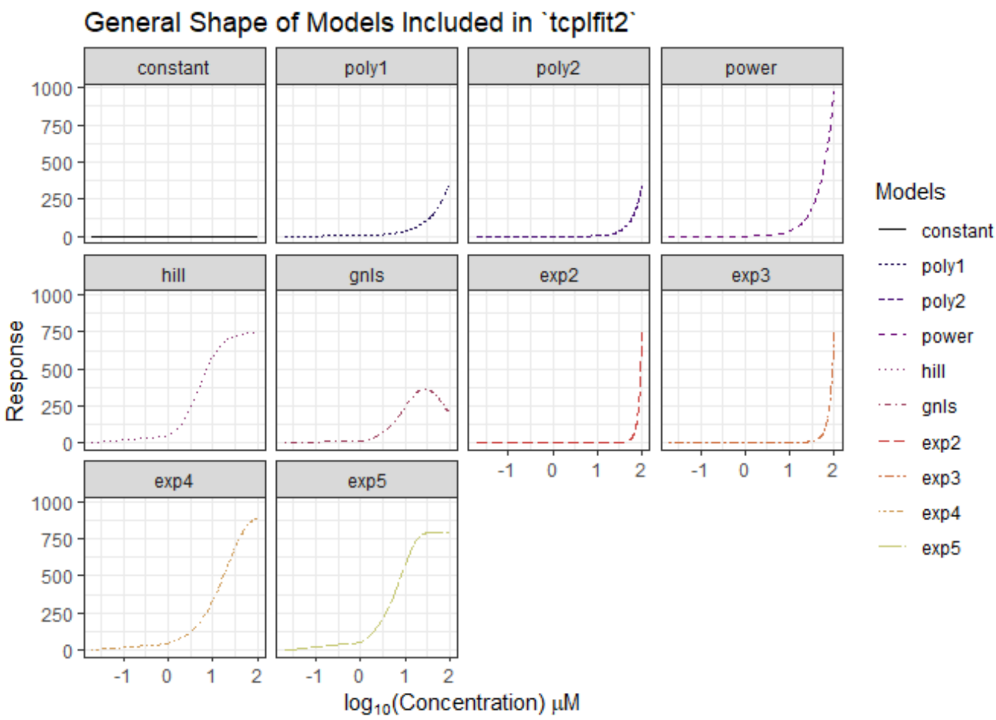
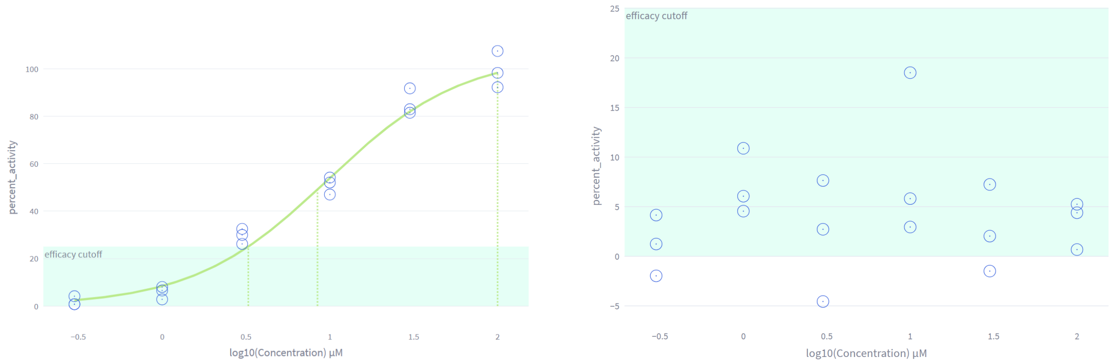


Figure 4.3: Employed curve-fit models in tcpl v3.0 for fitting concentration-response data series through the application of maximum likelihood estimation. Figure obtained from [28].



(a) POD estimates for the chemical *Picoxystrobin* (DTXSID9047542) tested in the assay endpoint with $aeid = 753$. The efficacy cutoff is defined at 25 percent-of-control activity. The winning fit model was the Hill function. *ACC*: The AC at the efficacy cutoff is at $3.3\mu M$. *AC50*: The AC at 50% of the maximum response is at $8.4\mu M$. *ACtop*: The AC at the maximum response is at $100\mu M$.

(b) POD estimates are not available for the chemical compound *PharmaGSID_48518* (DTXSID9048518) tested in the same assay endpoint as shown in the left figure. In this case, was unnecessary as no response reached or exceeded 80% of the efficacy cutoff, clearly indicating the inactivity of the compound. In such scenarios, a calculation of POD estimates is not applicable.

Figure 4.4: Presence Matrix: assay endpoint-compound relationship.

4.1.7 Hit Calling

The *continuous hitcall* is a measure of the probability that a compound is active (toxic), calculated based on the product of the following three probability values [27]:

i. that at least one median response is greater than the efficacy cutoff, computed by using the error parameter from the model fit and Student t-distribution to calculate the odds of at least one response exceeding the efficacy cutoff;

ii. that the top of the winning fitted curve is above the cutoff which is the likelihood ratio of the one-sided probability of the efficacy cutoff being exceeded;

iii. that the winning AIC value is less than that of the constant model:

$$\frac{e^{-\frac{1}{2}AIC_{\text{winning}}}}{e^{-\frac{1}{2}AIC_{\text{winning}}} + e^{-\frac{1}{2}AIC_{\text{cnst}}}} \tag{4.1}$$

In certain instances, compounds underwent multiple tests within a single assay endpoint, leading to their association with multiple CRS. In these exceptional cases, a hitcall is computed for each CRS, and then highest hitcall value is recorded as the compound's ultimate hitcall.

4.1.8 Flagging

Finally, after processing, each CRS is assigned to an appropriate fit category based on the level of certainty in the estimated bioactivity. Additionally, cautionary flags are assigned to account for problematic data series or or uncertainties related fits and hits.

4.2 New Toxicity Pipeline Implementation: pytcpl

4.2.1 Introduction

This thesis introduces pytcpl[4], a streamlined `Python` repository inspired by the `R` packages tcpl and tcplfit2. This package is crafted to accomodate cusomizable processing steps and facilitate interactive data visualization with an own *Curve Surfer*[5]. The package optimizes data storage and generates compressed `Parquet` files of the relevant raw data and metadata from *invitroDBv4.1*. Exclusively utilizing this repository eliminates the need for a complex and extensive database installation, rendering downstream analysis more accessible and efficient. It enables researchers who prefer `Python` to easily participate in data analysis and exploration, overcoming any limitations associated with using `R` code.

The pytcpl pipeline adds an additional setup and post-processing step around the main pipeline:

- **Setup**: This step involves user-specified subsetting of assay endpoints, tagging assays with external assay annotations, enabling workload balancing for distributed processing and generating `Parquet` files from all raw and metadata, optionally for database decoupled analysis.
- **Main** (similar to tcpl+tcplFit2): This step involves cutoff determination, curve fitting, hit calling and flagging.
- **Post-Processing**: This step has the goal of improving the overall quality of the data and involves post-processing curation, cytotoxicity interference reevaluation and the custom export of the final results.

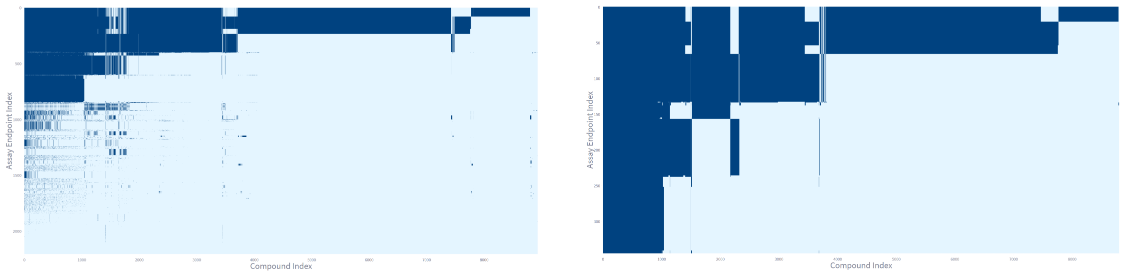
4.2.2 Setup step

Subsetting Data

For a better data comprehension, the presence matrix denoted as $P \in 0, 1^{m \times n}$ is introduced. In this matrix, rows (indexed by i) represent assay endpoints a_i, and columns (indexed by j) indicate whether testing was performed (1) or not performed (0) for compound c_j in those endpoints. Due to selective compound testing across different assay endpoints, matrix P is sparse. For a visual representation of the presence matrix P covering all assay endpoints and compounds in *invitroDBv4.1*, refer to Figure 4.5a.

[4] https://github.com/rbBosshard/pytcpl

[5] https://pytcpl.streamlit.app/



(a) The presence matrix P_{all}, covers all assay endpoints and compounds from *invitroDBv4.1*, totaling $m = 2205$ assay endpoints and $n = 8935$ compounds, excluding 606 compounds lacking molecular fingerprints. When $P_{\text{all}_{ij}} = 1$, there are 3 196 178 CRS available for analysis

(b) P_{subset} covers a specific subset of relevant assay endpoints and compounds considered for this thesis, totaling $m = 345$ assay endpoints and $n = 8804$ compounds. Assayy endpoints with less then 1000 tested compounds were omitted. When $P_{\text{subset}_{ij}} = 1$, there are 1 043 222 CRS available for analysis.

Figure 4.5: Presence Matrix: In both (a) and (b), structured by ranking according to the number of compounds associated with each assay endpoint, with compounds sorted in descending order of their occurrence frequency.

In this thesis, we exclusively considered assay endpoints that had been tested with a minimum of 1000 compounds. This selection criterion ensures the presence of adequate data for subsequent training of robust machine learning models. You can refer to Figure 4.5b for a visual representation of the presence matrix P which includes only this particular subset of assay endpoints. From this moment forward, we will refer to this specific subset as *the* dataset, which will be the focus of this thesis.

Exernal Assay Annotation

The assay endpoints were tagged with external annotations that involve the attributed toxcity endpoint, the type of mechanistic target and the mode of action.

The investigated assay endpoints are enriched with external annotations attributed by the Integrated Chemical Environment (ICE) [29], which provide valuable context and information about each endpoint. These annotations encompass the following aspects:

1. **Toxicity Endpoint**: This annotation specifies the type of toxicity or adverse effect associated with each assay endpoint, helping to clarify the specific aspect of toxicity under investigation.
2. **Mechanistic Target**: This annotation sheds light on the particular target mechanism or biological pathway being studied.
3. **Mode of Action**: The annotations also describe how the tested compounds interact with the mechanistic targets andprovides insights into the underlying biological processes or actions involved.

Table 4.3: pytcpl Model Updates

Model	Label	Equations[1]	Role in pytcpl
Exponential 3	exp3	$f(x) = a\left(\exp\left(\left(\frac{x}{b}\right)^p\right) - 1\right)$	Omitted
Gain-Loss 2	gnls2	$f(x) = \frac{tp}{1+\left(\frac{ga}{x}\right)^p}\exp\left(-qx\right)$	New

[1] Parameters: a: x-scale, b: y-scale p: (gain) power, q: (loss) power, tp: top, ga: gain AC50

4.2.3 Main step

The pytcpl main pipeline is similar to the `R`-based tcpl+tcplFit2 pipeline, with the exception of the curve fitting stage where the pytcpl pipeline made a notable modification by excluding a suboptimal model and including a novel model. The removal of Exponential 3 model was due to its suboptimal capability in fitting models within the original tcpl pipeline. Furthermore, an additional Gain-Loss 2 model was introduced during the curve-fitting stage. This secondary model has one fewer model parameter than the primary Gain-Loss 1 model, which helps mitigate the risk of overfitting CRS data. Table 4.3 provides an overview of these changes within the pytcpl pipeline for reference.

4.2.4 Post-Processing step

Post-Processing Curation

Following the ICE guidelines[6], quality filters were implemented to enhance the processed concentration-response series. This step introduces OMIT/PASS curation warning flags, which could be applied either based on assay endpoints or compound quality control criteria.

Cytotoxicity Interference Reevaluation

As previously discussed in Chapter 2, the assessment of compound toxicity can be complicated by the presence of non-specific cytotoxic responses. In this section, we delve into the exploration of a method for reevaluating the reported hitcall status of active compounds, considering the estimated extent of cytotoxicity interference. The cytotoxicity of a compound in a target assay endpoint may be assessed by comparing the activity concentration at the efficacy cutoff, represented as ACC_{target}, with that of its corresponding viability assay endpoint counterpart (as shown in Figure 4.6), referred to as ACC_{cyto}.

If no counterpart is available in the database, we presented in the following a statistical approach that allows for a cytotoxicity estimate. It uses the median ACC for the compound of interest across a set of assay endpoints dedicated for capturing the cytotoxicity

[6] https://ice.ntp.niehs.nih.gov/DATASETDESCRIPTION?section=cHTS

aeid	assay_component_endpoint_name	aid	assay_name	assay_function_type
26	APR_HepG2_CellLoss_24hr	3	APR_HepG2_24hr	viability
38	APR_HepG2_P-H2AX_24hr	3	APR_HepG2_24hr	signaling
40	APR_HepG2_p53Act_24hr	3	APR_HepG2_24hr	signaling
46	APR_HepG2_CellLoss_72hr	4	APR_HepG2_72hr	viability
58	APR_HepG2_P-H2AX_72hr	4	APR_HepG2_72hr	signaling
60	APR_HepG2_p53Act_72hr	4	APR_HepG2_72hr	signaling

Figure 4.6: Each assay endpoint has an assay identifier (aid) used to match it with its viability counterpart that assesses cell loss. In this example, *APR_HepG2_CellLoss_24hr* (aeid=26) corresponds to aeid=38 and aeid=40. Similarly, *APR_HepG2_CellLoss_72hr* (aeid=46) corresponds to aeid=58 and aeid=60.

burst. The ACC is assumed to have a Gaussian error distribution. Cytotoxicity in terms of the respective potencies is assumed when: $ACC_{\text{cyto}} \leq ACC_{\text{target}}$. The probability can be expressed as:

$$P(\text{cytotoxic}) = P(ACC_{\text{cyto}} - ACC_{\text{target}} \leq 0) = \Phi\left(\frac{ACC_{\text{cyto}} - ACC_{\text{target}}}{\sqrt{\text{SD}^2_{ACC_{\text{cyto}}} + \text{SD}^2_{ACC_{\text{target}}}}}\right)$$

where Φ is the Gaussian cumulative distribution function. The standard deviations $\text{SD}_{ACC_{\text{cyto}}}$ and $\text{SD}_{ACC_{\text{target}}}$ are unknown but are estimated as $0.3 \log_{10} \mu M$ units [30].

For the statistical approach with the cytotoxicity burst assays, $\text{SD}_{ACC_{\text{cyto}}}$ can be derived from the median absolute deviation (MAD) of the respective ACC values. Additionally, $P(\text{cytotoxic})$ is muliplied by $\frac{n_{\text{hit}}}{n_{\text{tested}}}$, the ratio of the number where the compound was considerd active divided by the number of cytotoxicity burst assay endpoints where the compound was tested.

Ultitmately, $P(\text{cytotoxic})$ is then mulitplied with the original continuous hitcall of active compounds. The final cytotoxicity-corrected hitcall is then defined as follows: $\text{hitcall}_{\text{c}} = \text{hitcall}_{\text{original}} * (1 - P(\text{cytotoxic}))$.

4.2.5 Curve Surfer

Figure 4.7 presents the developed *Curve Surfer*, a browser-based application that enables interactive data exploration and visualization of the processed data. The curve surfer tool is built using Streamlit, an open-source Python library that makes it easy to build custom web-apps for machine learning and data science.

4.3 Toxicity Prediction from Molecular Fingerprints: Machine Learning Pipeline

The primary goal is to develop individual machine learning models for each assay endpoint, enabling the prediction of assay-specific compound toxicity based on molecular

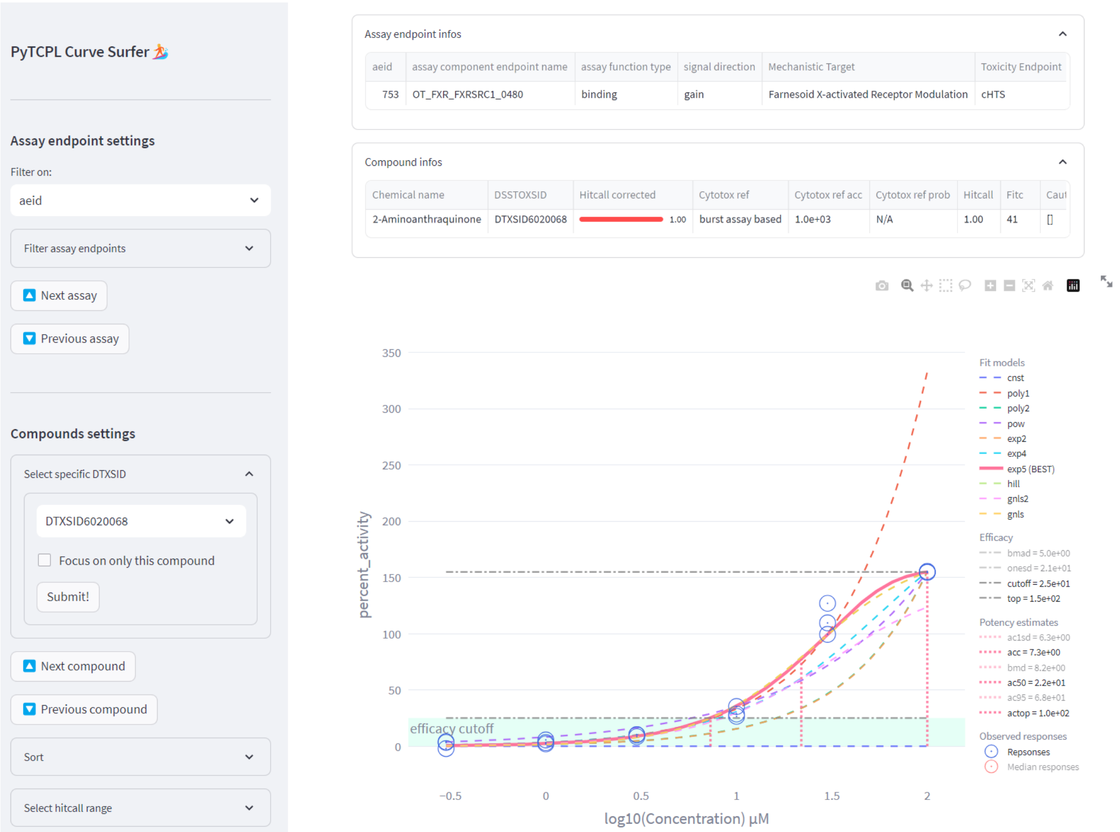


Figure 4.7: The curve surfer provides the capability to narrow down assay endpoints based on critical annotations, and compounds can be selectively filtered using their DTXSID. Users can navigate through assay endpoints or the compounds within the current assay endpoint. Additionally, compounds can be filtered by their hitcall value or POD estimates using a range slider. Subsequently, the curve surfer displays comprehensive details for the chosen compound within the opted assay endpoint, showcasing CRS data along with curve fit models and metadata.

structure inputs. These models utilize molecular fingerprints to predict compound toxicity, as illustrated in Figure 4.8. Please take into consideration that we utilized both binary classification and regression models. In the case of binary classification, we applied a threshold of 0.5 to convert the continuous hitcall target values into binary outcomes.

To create these individual datasets, we extract compounds that possess toxicity data for a given assay endpoint from the outputs generated by pytcpl. The associated hitcall values for these compounds are used as target variables within the machine learning model.

The binary input features for the model consist of molecular fingerprints with 2362 bits derived from chemical structures. The structural data was obtained from the U.S. EPA's DSSTox database, accessed through the CompTox Chemicals Dashboard. The

Index	X (fingerprint features)						y (activity label)
Compound ID	fps 1	fps 2	fps 3	fps 4	...	fps n	hitcall
DTXSID 1	0	1	0	0	...	0	0.00
DTXSID 2	1	0	0	0	...	1	0.01
DTXSID 3	0	0	1	1	...	0	0.00
DTXSID 4	1	0	0	0	...	0	0.98
...	...	...	...	...	...	...	...
DTXSID m	0	0	1	0	...	1	0.01

Figure 4.8: Schematic example of a machine learning dataset related to a single assay endpoint. The dataset is structured into a feature matrix with $n = 2362$ and a target vector. The feature matrix consists of molecular fingerprints, and the target vector is the hitcall value. For binary classification, the hitcall value is binarized based on a specific activity threshold.

structural data mining and the necessary structure cleanup, as mentioned in Section 2.1, was conducted by Dr. Kasia Arturi[7].

The machine learning pipeline is structured into three main stages: model training, model evaluation and model application. The following sections and the Figure illustrated in Figure 4.9 provide a detailed description of each stage.

4.3.1 Training

The train stage is summarized in Figure 4.10 and involves the generation of individual machine learning models for each assay endpoint. Each model is trained on a subset of the dataset with a 80/20 train/validation split.

Feature Selection

For all models we do feature selection based on machine learning model that extracts the most important features. This is then used as a transformer on the input data. The XGBoost model was used for this purpose. The number of features to be selected was determined by the number of features that reach the mean feature importance.

Model Selection

The following supervised machine learning models from the `scikit-learn` library were considered for this thesis:

1. **Logistic Regression** is a linear model that utilizes the logistic function to model binary dependent variables. It serves as a straightforward and interpretable model, often employed as a baseline for binary classification tasks.

[7] https://gitlab.renkulab.io/expectmine/generating-fingerprints

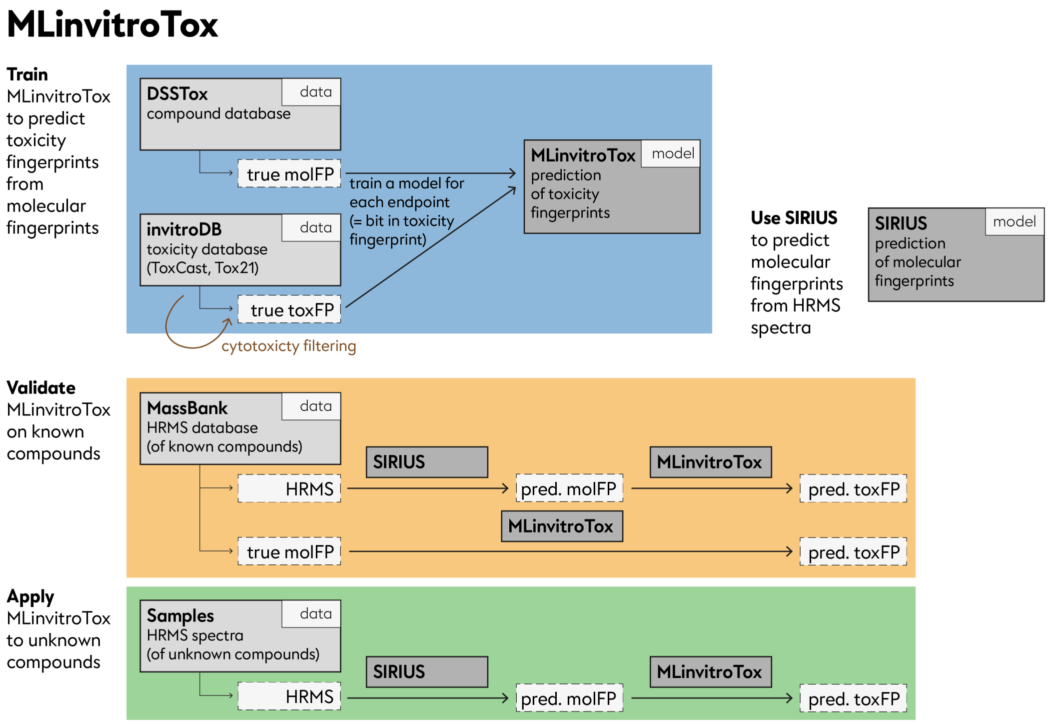


Figure 4.9: MLinvitroTox: Machine Learning Pipeline Steps. Figure created by Lili Gasser.

2. **Support Vector Machine**: is a robust model with a lower susceptibility to overfitting and the ability to handle high-dimensional feature spaces.
3. **Random Forest** is a bagging (bootstrap aggregating) ensemble learning technique in machine learning that constructs a multitude of decision trees during training and combines their predictions, resulting in robust and accurate models with the advantage of reduced overfitting and the ability to handle high-dimensional data.
4. **XGBoost** is a gradient boosting ensemble learning technique that combines multiple weak learner decision trees sequentially, with each new learner giving more weight to the examples that the previous learners struggled with. It provides typically high predictive accuracy and efficiency through techniques like gradient optimization and regularization.
5. **Multi-Layer Perceptron** is a type of artificial neural network that consists of multiple layers of interconnected neurons and is used for various machine learning tasks, offering the advantage of modeling complex non-linear relationships in data.

For every machine learning model, the selection process is based on a grid search over

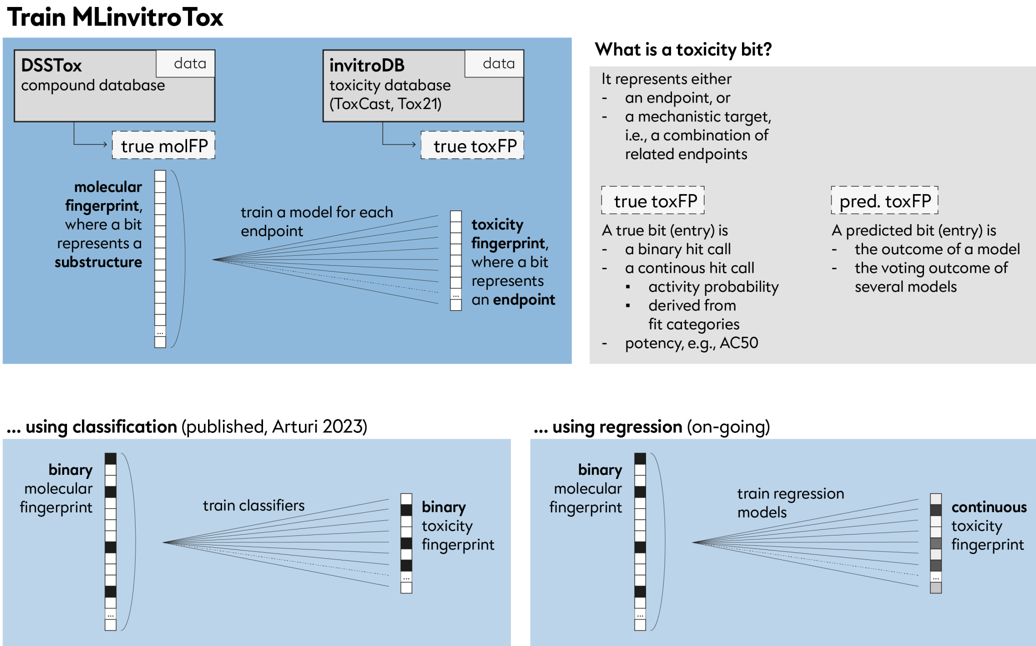


Figure 4.10: MLinvitroTox Train Step. Figure created by Lili Gasser.

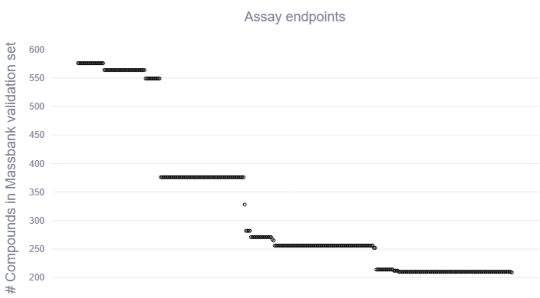
a set of hyperparameters, using 5-fold cross-validation. The hyperparameters, specified in a seperate config file, are optimized for binary classification based on the F_β score, a generalization of the F_1. The F_1-score is the harmonic mean of the precision and recall and the more generic F_β score applies additional weights, valuing one of precision or recall more than the other. We set $\beta = 2$ to value recall more than precision.

4.3.2 Evaluation

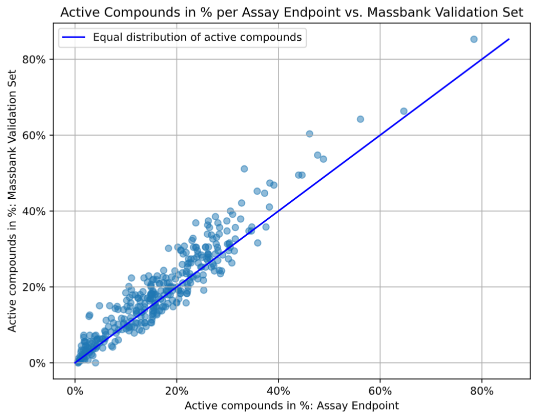
To assess the performance of our trained models, we used two separate validation sets that were not part of the training data:

The first straightforward validation set, was utilized to evaluate how well the the best estimator found by the grid search 5-fold cross-validation generalizes for unseen compounds. This set was randomly sampled from the tested compounds in the specific assay endpoints, ensuring that the number of active and inactive compounds was balanced.

The MassBank validation set, serving as the second validation set, was utilized to evaluate the model's generalization capabilities, specifically examining the disparity



(a) The Overlap between compounds for that we have MassBank spectra data and compounds we have toxicity data. The number of compounds in the MassBank validation set varies for different assay endpoints due to differences in the overlap between the compounds tested in MassBank spectra data and those present in the toxicity data.



(b) Plotting the ratio of active (binarized) hitcall values for all compounds tested in the assay endpoint against the ratio of active (binarized) hitcall values for the compounds in the MassBank validation set. The line represents the ideal case where the ratios are equal and the validation set is representative of the entire dataset.

Figure 4.11: MassBank validation set.

between chemical structure space and fragmentation spectra. This evaluation is pivotal as it assesses the model's performance during its application stage. Prior to any further data splitting, this subset of compounds was separated. This subset includes compounds for which we have access to both actual and SIRIUS predicted fingerprints originating from MassBank spectra data. The availability of both sets of fingerprints enables us to assess the reliability of the predicted fingerprints as indicators of compound toxicity. This assessment is carried out by comparing the models' performance on both the actual and predicted fingerprints.

It is worth noting that potentially data-leaking compounds were excluded that were used in training the SIRIUS+CSI:FingerID prediction model itself, leaving us with a maximum of 315 compounds that can be safely used for MassBank validation. However, the MassBank validation set's size varies for different assay endpoints due to differences in the overlap between the compounds tested in MassBank spectra data and those present in the toxicity data, despite the fixed number of compounds available in MassBank spectra data, illustrated in Figure 4.11. Moreover the the representativenes of the validation set is illustrated in Figure 4.11b.

4.3.3 Application

The presence of distinct prediction models for each assay endpoint enables the grouping of these endpoints based on their annotations, such as the biological process or the mechanistic target annotation. This ultimately results in toxicity predictions averaged within these groups. These collective toxicity predictions are referred to as *toxicity fingerprint* which serve the purpose of identifying the most toxic compounds for each specific assay endpoint.

Chapter 5

Results and Discussion

5.1 Results

When evaluating the effectiveness of a binary prediction technique using a validation dataset with known activities, four central values are considered:

1. True Positives (TP): The number of correctly predicted active cases.
2. True Negatives (TN): The number of correctly predicted inactive cases.
3. False Positives (FP): The number of incorrectly predicted active cases.
4. False Negatives (FN): The number of incorrectly predicted inactive cases.

Using these four values, it is possible to construct a confusion matrix and derive evaluation metrics, as illustrated in Figure 5.1:

5.1.1 Evaluation

5.2 Discussion

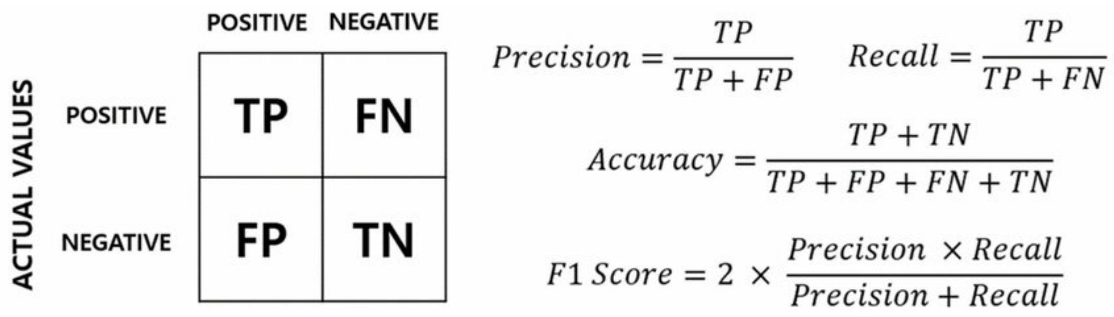


Figure 5.1: Confusion Matrix and Metrics: Accuracy, Precision, Recall, and F1 score. Figure obtained from [31].

Chapter 6

Conclusion

Bibliography

[1] U. N. E. Programme, *Global chemicals outlook ii - from legacies to innovative solutions: Implementing the 2030 agenda for sustainable development - synthesis report*, 2019. [Online]. Available: https://wedocs.unep.org/20.500.11822/27651.

[2] C. A. Service, *Chemical abstracts service (cas) is a division of the american chemical society*, Source of chemical information located in Columbus, Ohio, United States, https://www.cas.org/support/documentation/cas-databases, 2023.

[3] R. Schwarzenbach *et al.*, "The challenge of micropollutants in aquatic systems," *Science (New York, N.Y.)*, vol. 313, pp. 1072–7, Sep. 2006. DOI: 10.1126/science.1127291.

[4] E. Commission, D.-G. for Research, and Innovation, *European Green Deal - Research & innovation call*. Publications Office of the European Union, 2021. DOI: 10.2777/33415.

[5] E. Commission, "Eu chemicals strategy for sustainability towards a toxic-free environment," 2020, Communication from the Commission to the European Parliament, the Council, the European Economic and Social Committee and the Committee of the Regions - Chemicals Strategy for Sustainability Towards a Toxic-Free Environment. [Online]. Available: https://environment.ec.europa.eu/strategy/chemicals-strategy_en.

[6] S. Tamara, M. A. den Boer, and A. J. R. Heck, "High-resolution native mass spectrometry," *Chemical Reviews*, vol. 122, no. 8, pp. 7269–7326, 2022, PMID: 34415162. DOI: 10.1021/acs.chemrev.1c00212. eprint: https://doi.org/10.1021/acs.chemrev.1c00212. [Online]. Available: https://doi.org/10.1021/acs.chemrev.1c00212.

[7] J. Hollender, E. L. Schymanski, H. P. Singer, and P. L. Ferguson, "Nontarget screening with high resolution mass spectrometry in the environment: Ready to go?" *Environmental Science & Technology*, vol. 51, no. 20, pp. 11 505–11 512, 2017, PMID: 28877430. DOI: 10.1021/acs.est.7b02184. eprint: https://doi.org/10.

1021/acs.est.7b02184. [Online]. Available: https://doi.org/10.1021/acs.est.7b02184.

[8] P. Banerjee, A. O. Eckert, A. K. Schrey, and R. Preissner, "ProTox-II: a webserver for the prediction of toxicity of chemicals," *Nucleic Acids Research*, vol. 46, no. W1, W257–W263, Apr. 2018, ISSN: 0305-1048. DOI: 10.1093/nar/gky318. eprint: https://academic.oup.com/nar/article-pdf/46/W1/W257/25110434/gky318.pdf. [Online]. Available: https://doi.org/10.1093/nar/gky318.

[9] N. H. G. R. I. Maggie Bartlett. "Chemical genomics robot." (2009), [Online]. Available: https://en.wikipedia.org/wiki/High-throughput_screening#/media/File:Chemical_Genomics_Robot.jpg.

[10] J. Rudd. "High throughput screening - accelerating drug discovery efforts." (2017), [Online]. Available: https://www.ddw-online.com/hts-a-strategy-for-drug-discovery-900-200008/.

[11] K. Arturi and J. Hollender, "Machine learning-based hazard-driven prioritization of features in nontarget screening of environmental high-resolution mass spectrometry data," *Environmental Science & Technology*, vol. 0, no. 0, null, 0, PMID: 37279189. DOI: 10.1021/acs.est.3c00304. eprint: https://doi.org/10.1021/acs.est.3c00304. [Online]. Available: https://doi.org/10.1021/acs.est.3c00304.

[12] K. Dührkop *et al.*, "Sirius 4: A rapid tool for turning tandem mass spectra into metabolite structure information," *Nature methods*, vol. 16, no. 4, pp. 299–302, Apr. 2019, ISSN: 1548-7091. DOI: 10.1038/s41592-019-0344-8. [Online]. Available: https://research.aalto.fi/files/32997691/SCI_Duhrkop_Fleischauer_Sirius_4_Turning_tandem.pdf.

[13] *Massbank: High quality mass spectral database*, https://massbank.eu/MassBank/, Accessed: 2023.

[14] T. Janela, K. Takeuchi, and J. Bajorath, "Introducing a chemically intuitive core-substituent fingerprint designed to explore structural requirements for effective similarity searching and machine learning," *Molecules*, vol. 27, no. 7, 2022, ISSN: 1420-3049. DOI: 10.3390/molecules27072331. [Online]. Available: https://www.mdpi.com/1420-3049/27/7/2331.

[15] S. M. Bell *et al.*, "In vitro to in vivo extrapolation for high throughput prioritization and decision making," *Toxicology in Vitro*, vol. 47, pp. 213–227, 2018, ISSN: 0887-2333. DOI: https://doi.org/10.1016/j.tiv.2017.11.016. [Online]. Available: https://www.sciencedirect.com/science/article/pii/S0887233317303661.

[16] P. Nymark *et al.*, "Systematic organization of covid-19 data supported by the adverse outcome pathway framework," *Frontiers in Public Health*, vol. 9, May 2021. DOI: 10.3389/fpubh.2021.638605.

[17] R. Judson *et al.*, "Editor's Highlight: Analysis of the Effects of Cell Stress and Cytotoxicity on In Vitro Assay Activity Across a Diverse Chemical and Assay Space," *Toxicological Sciences*, vol. 152, no. 2, pp. 323–339, May 2016, ISSN: 1096-6080. DOI: 10.1093/toxsci/kfw092. eprint: https://academic.oup.com/toxsci/article-pdf/152/2/323/26290632/kfw092.pdf. [Online]. Available: https://doi.org/10.1093/toxsci/kfw092.

[18] B. Escher, P. Neale, and F. Leusch, *Bioanalytical Tools in Water Quality Assessment*. IWA Publishing, Jun. 2021, ISBN: 9781789061987. DOI: 10.2166/9781789061987. eprint: https://iwaponline.com/book-pdf/899726/wio9781789061987.pdf. [Online]. Available: https://doi.org/10.2166/9781789061987.

[19] K. A. Fay *et al.*, "Differentiating Pathway-Specific From Nonspecific Effects in High-Throughput Toxicity Data: A Foundation for Prioritizing Adverse Outcome Pathway Development," *Toxicological Sciences*, vol. 163, no. 2, pp. 500–515, Feb. 2018, ISSN: 1096-6080. DOI: 10.1093/toxsci/kfy049. eprint: https://academic.oup.com/toxsci/article-pdf/163/2/500/24935129/kfy049.pdf. [Online]. Available: https://doi.org/10.1093/toxsci/kfy049.

[20] C. N. Cavasotto and V. Scardino, "Machine learning toxicity prediction: Latest advances by toxicity end point," *ACS Omega*, vol. 7, no. 51, pp. 47 536–47 546, 2022. DOI: 10.1021/acsomega.2c05693. eprint: https://doi.org/10.1021/acsomega.2c05693. [Online]. Available: https://doi.org/10.1021/acsomega.2c05693.

[21] A. M. Richard *et al.*, "The tox21 10k compound library: Collaborative chemistry advancing toxicology," *Chemical Research in Toxicology*, vol. 34, no. 2, pp. 189–216, 2021, PMID: 33140634. DOI: 10.1021/acs.chemrestox.0c00264. eprint: https://doi.org/10.1021/acs.chemrestox.0c00264. [Online]. Available: https://doi.org/10.1021/acs.chemrestox.0c00264.

[22] F. Kretschmer, J. Seipp, M. Ludwig, G. W. Klau, and S. Böcker, "Small molecule machine learning: All models are wrong, some may not even be useful," *bioRxiv*, 2023. DOI: 10.1101/2023.03.27.534311. eprint: https://www.biorxiv.org/content/early/2023/03/27/2023.03.27.534311.full.pdf. [Online]. Available: https://www.biorxiv.org/content/early/2023/03/27/2023.03.27.534311.

[23] P. Peets, W.-C. Wang, M. MacLeod, M. Breitholtz, J. W. Martin, and A. Kruve, "Ms2tox machine learning tool for predicting the ecotoxicity of unidentified chemicals in water by nontarget lc-hrms," *Environmental Science & Technology*, vol. 56, no. 22, pp. 15 508–15 517, 2022, PMID: 36269851. DOI: 10.1021/acs.est.2c02536. eprint: https://doi.org/10.1021/acs.est.2c02536. [Online]. Available: https://doi.org/10.1021/acs.est.2c02536.

[24] A. J. Williams *et al.*, "The comptox chemistry dashboard: A community data resource for environmental chemistry," *Journal of Cheminformatics*, vol. 9, no. 1, p. 61, 2017. DOI: 10.1186/s13321-017-0247-6. [Online]. Available: https://doi.org/10.1186/s13321-017-0247-6.

[25] L. Wu, R. Huang, I. V. Tetko, Z. Xia, J. Xu, and W. Tong, "Trade-off predictivity and explainability for machine-learning powered predictive toxicology: An in-depth investigation with tox21 data sets," *Chemical Research in Toxicology*, vol. 34, no. 2, pp. 541–549, 2021, PMID: 33513003. DOI: 10.1021/acs.chemrestox.0c00373. eprint: https://doi.org/10.1021/acs.chemrestox.0c00373. [Online]. Available: https://doi.org/10.1021/acs.chemrestox.0c00373.

[26] J. Phuong *et al.* "Toxcast assay annotation data user guide." (2014), [Online]. Available: https://www.epa.gov/sites/default/files/2015-08/documents/toxcast_assay_annotation_data_users_guide_20141021.pdf.

[27] T. Sheffield, J. Brown, S. Davidson, K. P. Friedman, and R. Judson, "tcplfit2: an R-language general purpose concentration–response modeling package," *Bioinformatics*, vol. 38, no. 4, pp. 1157–1158, Nov. 2021, ISSN: 1367-4803. DOI: 10.1093/bioinformatics/btab779. eprint: https://academic.oup.com/bioinformatics/article-pdf/38/4/1157/50422999/btab779.pdf. [Online]. Available: https://doi.org/10.1093/bioinformatics/btab779.

[28] C. for Computational Toxicology and U. E. Exposure, *Tcpl v3.0 data processing*, R package vignette for the tcpl package v3.0, CRAN, 2023. [Online]. Available: https://cran.r-project.org/web/packages/tcpl/vignettes/Data_processing.html.

[29] A. B. Daniel *et al.*, "Data curation to support toxicity assessments using the integrated chemical environment," *Frontiers in Toxicology*, vol. 4, 2022, ISSN: 2673-3080. DOI: 10.3389/ftox.2022.987848. [Online]. Available: https://www.frontiersin.org/articles/10.3389/ftox.2022.987848.

[30] E. D. Watt and R. S. Judson, "Uncertainty quantification in toxcast high throughput screening," *PLOS ONE*, vol. 13, no. 7, pp. 1–23, Jul. 2018. DOI: 10.1371/journal.pone.0196963. [Online]. Available: https://doi.org/10.1371/journal.pone.0196963.

[31] D. Seol, J. Choi, C. Kim, and S. Hong, "Alleviating class-imbalance data of semiconductor equipment anomaly detection study," *Electronics*, vol. 12, p. 585, Jan. 2023. DOI: 10.3390/electronics12030585.

Appendix A

Appendix

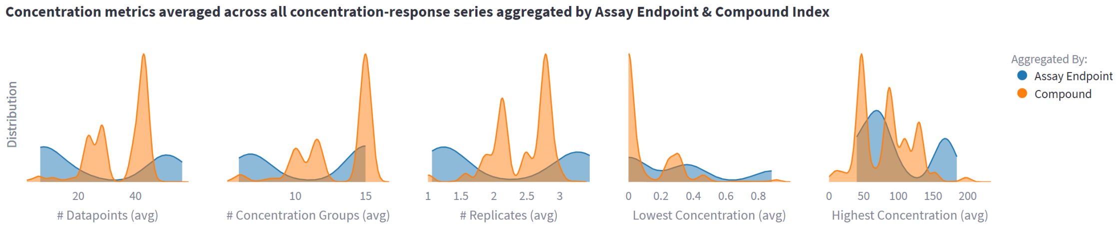


Figure A.1: Concentration metrics averaged across all concentration-response series aggregated by assay endpoint (blue) and compound (orange). E.g., the first chart shows the distribution (blue) on the average number of datapoints across all assay enpoint $a_i \in A$ with $\frac{1}{|C_i|}\sum_j n_{\text{datapoints}_{i,j}}$ and across all compounds $c_j \in C$ with $\frac{1}{|A_j|}\sum_i n_{\text{datapoints}_{i,j}}$. Similarly, the process is repeated for the other metrics: $n_{\text{groups}_{i,j}}$, $n_{\text{replicates}_{i,j}}$, $min_{\text{conc}_{i,j}}$, and $max_{\text{conc}_{i,j}}$.

Table A.1: Assay Source Names and Long Names

assay_source_name	assay_source_long_name
ACEA	ACEA Biosciences
APR	Apredica
ATG	Attagene
BSK	Bioseek
NVS	Novascreen
OT	Odyssey Thera
TOX21	Tox21/NCGC
CEETOX	Ceetox/OpAns
LTEA	LifeTech/Expression Analysis
VALA	VALA Sciences
CLD	CellzDirect
CCTE_PADILLA	CCTE Padilla Lab
TANGUAY	Tanguay Lab
STM	Stemina Biomarker Discovery
ARUNA	ArunA Biomedical
CCTE	CCTE Labs
CCTE_SHAFER	CCTE Shafer Lab
CPHEA_STOKER	CPHEA Stoker and Laws Labs
CCTE_GLTED	CCTE Great Lakes Toxicology and Ecology Division
UPITT	University of Pittsburgh Johnston Lab
UKN	University of Konstanz
ERF	Eurofins
TAMU	Texas A&M University
IUF	Leibniz Research Institute for Environmental Medicine
CCTE_MUNDY	CCTE Mundy Lab
UTOR	University of Toronto, Peng Laboratory